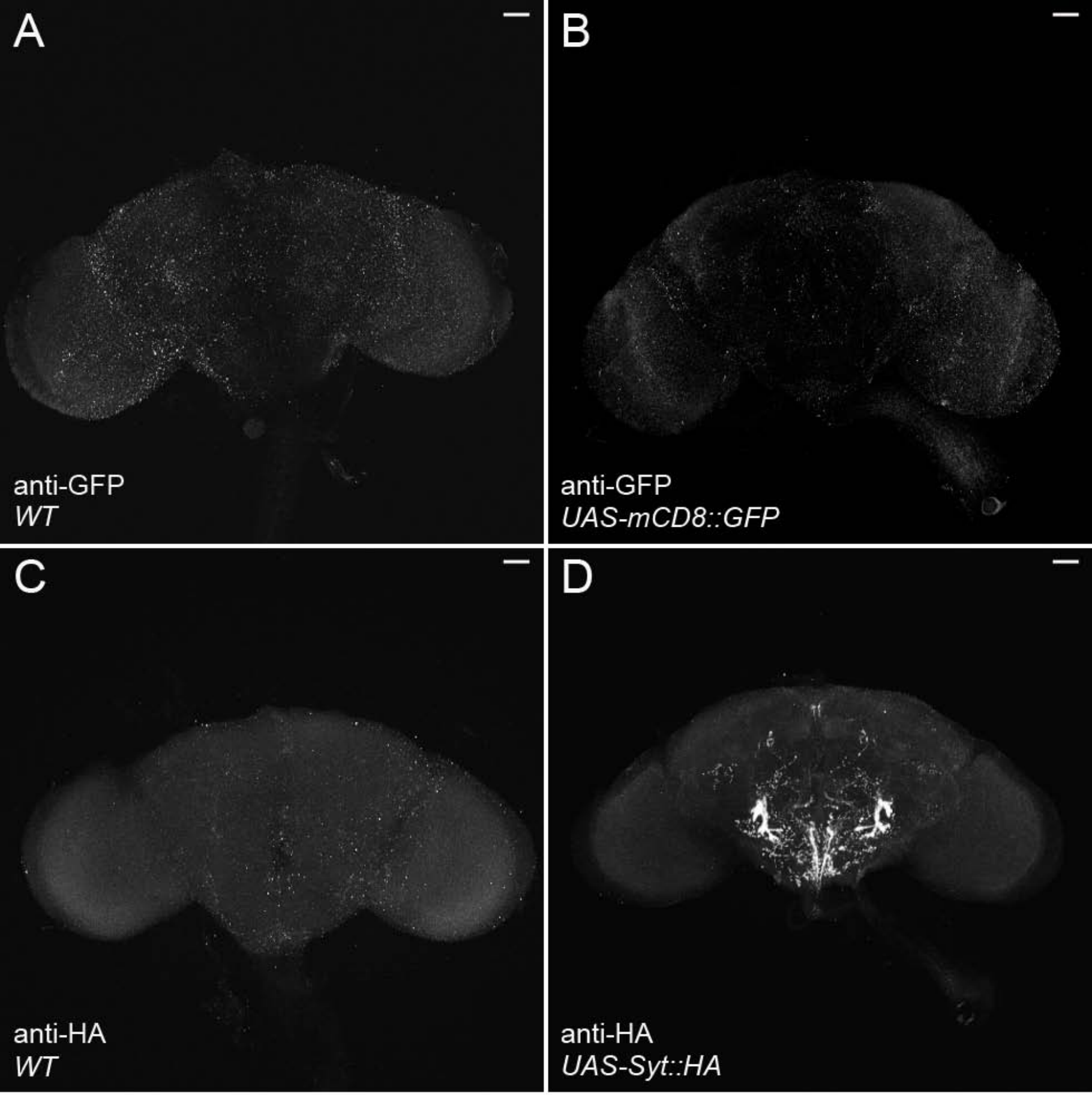
Supplementary Fig. 1. Specificity of primary antibodies anti-GFP (A), and anti-HA (C) in the wild-type brains. GAL4-independant expression of the reporter constructs UAS-mCD8::GFP (B), and UAS-Syt-HA (D). Panels A-D show projections of stacks through the entire brain. There is no detectable signal in wild-type brains (WT) stained with anti-GFP or anti-HA (A and C). B: The signal of the anti-GFP antibody was undetectable in the flies carrying UAS-mCD8::GFP in the absence of GAL4. D: Utilizing the anti-HA antibody, we detected GAL4-independent expression of UAS-Syt-HA in the subesophageal ganglion, the ventromedial protocerebrum and the antennal nerve. Scale bar = 25 um.

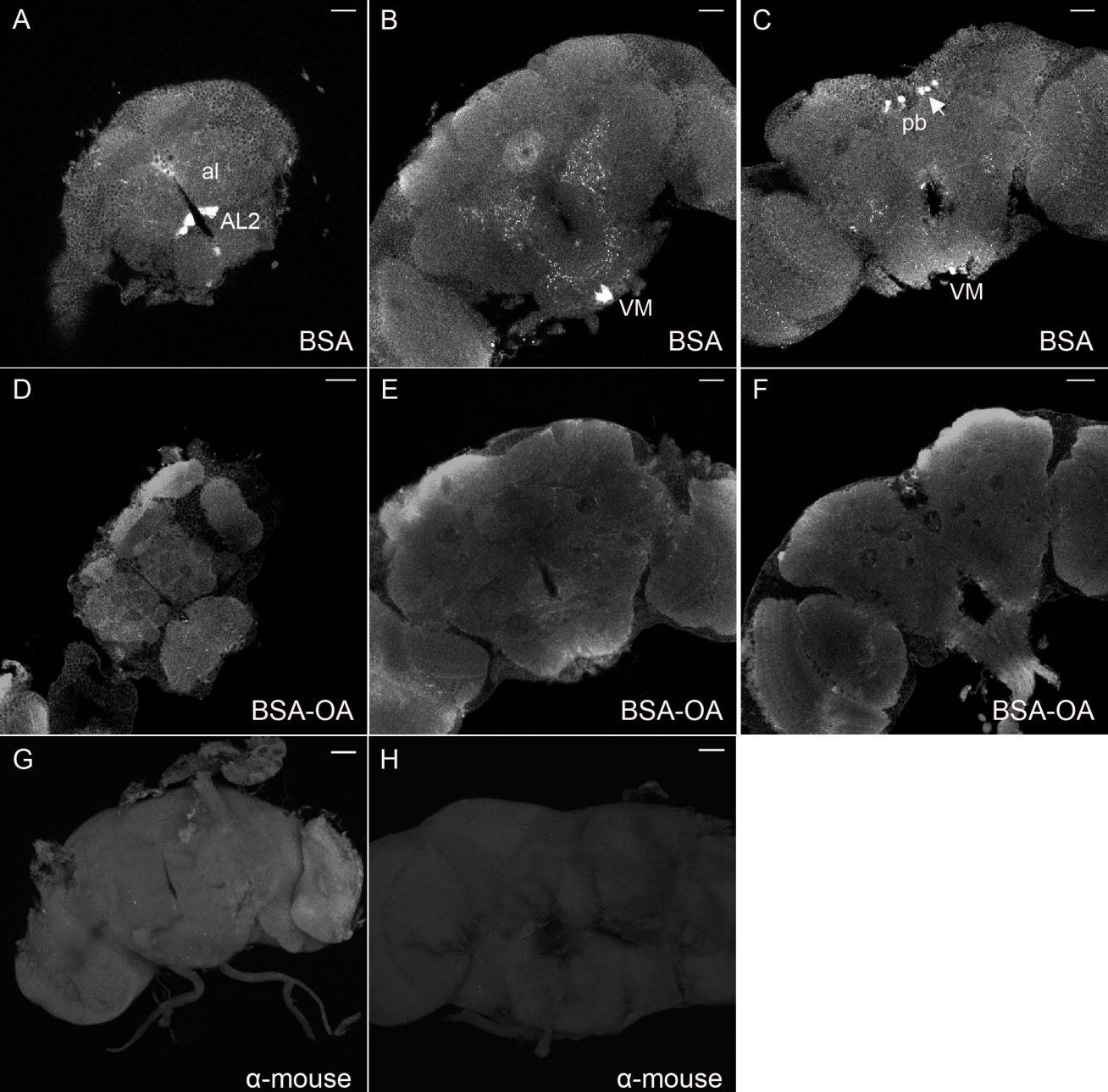
Supplementary Fig. 2. Specificity of the anti-OA antibody. A-F: Single optical sections of wild-type brains. The anti-OA antibody was preincubated with BSA (A-C) and OA conjugated to BSA (D-F). OA signal is monitored in white. Panels A-C and D-F show comparable regions in the brain. A-C: After preincubating the anti-OA antibody with BSA there is signal detectable in cell clusters located at the antennal lobes (AL2;A), along the ventral midline (VM; B and C) and at the protocerebral bridge (arrow; C). D-F: Preincubation of the antibody with OA conjugated to BSA abolishes the OA-signal. Unspecific staining of the anti-OA antibody was repeatedly observed in the cortical area. G and H: Projection of the anterior (G) and the posterior (H) brain stained without the primary antibody. Under these conditions the unspecific signal in the cortical area is less pronounced. Scale bar = 25 um.

Supplementary Fig. 3. Comparison of the anti-OA and anti-TA antibodies. The signals of the anti-OA and anti-TA antibodies are visualized in magenta and green, respectively. A, B and C show projections of stacks, whereas D shows a single optical section. A-B: Confocal projections of the central brain. The OA and TA signals overlap in the AL2, VM, VL and PB1 clusters. All cells recognized by the OA antibody were also stained by the TA antibody. C: Magnification of the AL clusters. TA signal was observed in the AL1 cluster (arrowheads). D: Magnification of the PB1 cluster. The arrowheads show cells strongly labeled by the anti-TA, but not the anti-OA, antibody. Scale bar = 25 um.

Supplementary Fig. 4. Cell clusters in NP7088. Neuropils and membranes of GAL4-expressing cells are visualized by alpha-Synapsin (orange) and mCD8::GFP (white), respectively. A: A frontal view of a confocal projection of an entire central brain Clusters VM and AL2 were also observed in tdc2-GAL4. Weakly stained somata lateral to the SOG (cluster SOG; arrow; arrowhead in B) and a cluster on the middle inferiolateral protocerebrum (MIL) are specific for NP7088, but not OA-immunoreactive. B: Projection of confocal stacks of the middle to posterior central brain. The arrow indicates cells of the MSM cluster on the middle superior medial protocerebrum. C: Projection of confocal stacks of the posterior brain. The arrow indicates the cluster on the posterior lateral protocerebrum (PL). Scale bar = 25um



Suppl. Fig. 1, Busch et al.



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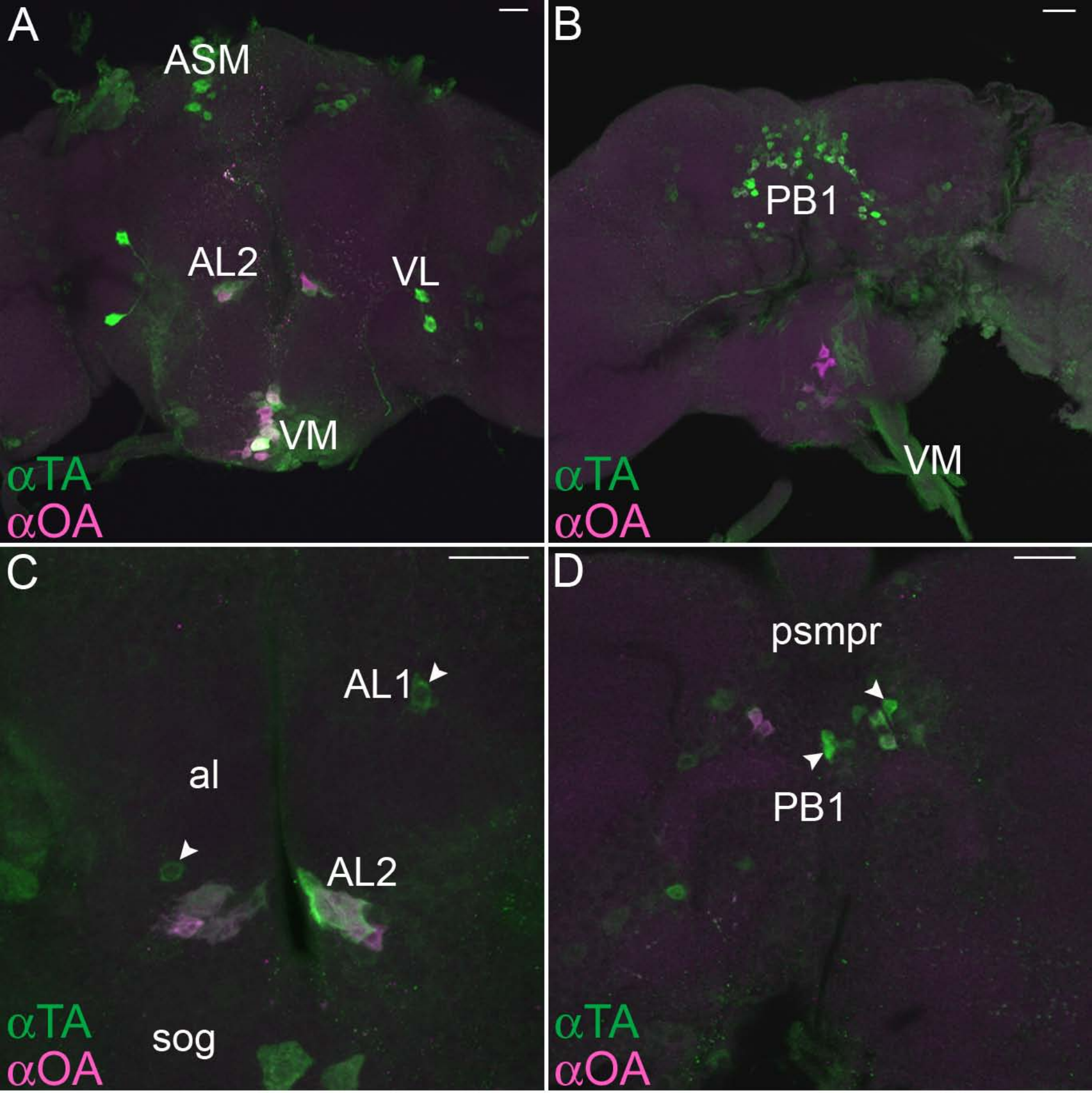

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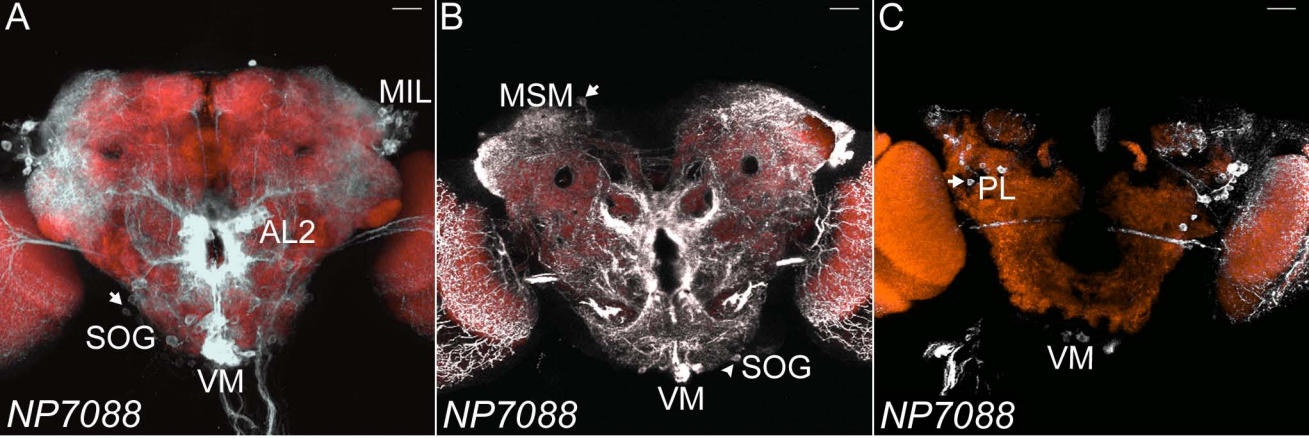

Suppl. Fig. 4, Busch et al.